NERRS Science Collaborative Progress Report for the Period 03/01/11 through 08/31/11
Project Title: *Legacy effects of land-use change and nitrogen source shifts on a benchmark system: Building capacity for collaborative research leadership at the Grand Bay Reserve*

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Project start date: 09/15/10

Report compiled by: R. H. Carmichael (PI)

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A. Progress overview: State the overall goal of your project, and briefly summarize in one or two paragraphs, what you planned to accomplish during this period and your progress on tasks for this reporting period. This overview will be made public for all reports, including confidential submissions.

Research goal

To measure land-use related N source and pathogen changes through time and define the resulting effects on ecosystem and human health in Grand Bay, AL by combining data from land-use models, sediment cores, modern sediment and water samples, ancient shell middens, living native and transplanted bivalves, and environmental attributes that cover time periods from up to 3000 years before present to 2020 for three subwatersheds and their receiving waters.

Planned activities and anticipated accomplishments

For this term (Q3 & Q4), we planned to continue land-use research and modeling efforts; begin processing midden samples and shell dating; take and process sediment cores; begin regular native bivalve and estuarine attribute (YSI, water and sediment) sampling; conduct stable isotope and microbial analyses on water, sediments, and oysters; continue sampling relevant wastewater sources; begin developing methods for oyster growth and taking growth measurements; regularly update our Facebook page and discussion board; share data at at least one meeting (of researchers, managers and/or stakeholders); and continue training students and technicians on technical methods (particularly stable isotope and microbial analyses).

B. Working with Intended Users:

- Describe the progress on tasks related to the integration of intended users into the project for this reporting period.
- What did you learn? Have there been any unanticipated challenges or opportunities?
- Who has been involved?

1. We conducted one core stakeholder meeting 0n 4 March 2011 at the Grand Bay NERR, where PI, Co-Is, Integration Lead, associated NERR staff, graduate student researcher, and project manager, Kalle Matso, reviewed project goals and changes to-date, brain-stormed for opportunities to a) increase participation by existing end-user participants and b) reach out to additional potential end users. We answered stakeholder questions, identified new ways for them to participate and potential new products. There was extensive discussion about the uses and applications of the project results. Specifically, Ed Jackson indicate his desire to include the project results in virtual tours of the area's archaeological sites, and Kathy Wilkinson expressed a desire to incorporate the information into her kayak tours, working closely with GBNERR. (meeting arranged by Carmichael and Walton; lead by Walton).

2. Integration Lead, Walton and graduate student Beth Condon (and PI Carmichael, to a lesser degree) maintained the project Facebook Page: http://www.facebook.com/pages/Grand-Bay-National-Estuarine-Research-Reserve-Science-Collaborative/153046948084497 (open to the public)

   Current 'likes' count of 53, with over 2,500 views of posts and 13 feedback posts in the month of August. Posts include research updates and images, particularly of graduate student Condon, FDA, and NERR field collaborators. This venue has proven useful to update participants and recognize and show appreciation for their efforts.

3. 14 March and 4 April, we conducted project review calls with Program Manager, Kalle Matso. The April call included participation by Walton, Carmichael, and Ruple to review the project to date. The primary suggestion was to "push the envelope" with regard to stakeholder participation and move past the focused work with core participants and deliver our message more broadly to the community (within the boundaries of the existing budget).

4. On 28 April, PI Carmichael and graduate student Condon met with EPA Ecologist, John Lehrter, to discuss our project and relationships to an ongoing EPA-collaborative project with Dr. Just Cebrian at DISL. Discussion focused on complementary coring efforts between our projects and whether we should adopt the EPA's coring protocol so that our data will be comparable to EPA collected data. We agreed to contact the University of West Florida to determine if using their vibracorer would be possible. We also discussed our ability to fill data gaps, particularly for microbial sampling down core, which are not possible for EPA core samples.

5. Auburn University Shellfish Laboratory (AUSL) provided aquacultured oysters for transplants, and Condon worked with MS DMR to obtain permits for the transplant work.

6. To meet the goals set in our NSC conference call (#3 above) and build on our initial outreach to the broader community,
    a. PI Carmichael met with Alabama Marine Resources newly instated Director, Chris Blankenship, in early May. Because Blankenship was not able to attend the initial stakeholder meeting, we used this meeting to discuss the project overview and how our project can meet Alabama's resource needs. Particular focus was given to the Bayou La Batre Wastewater Treatment plant and effects of outputs to the system adjacent to and immediately within Grand Bay at the Alabama-Mississippi border. Blankenship provided guidance for collecting sediment cores in the area and expressed interest in attending future stakeholder meetings.

    b. Grand Bay NERR Director and Co-I, Ruple, coordinated a stakeholder meeting to held in tandem with a meeting already planned for a separately funded (EPA-DISL) project ongoing at the NERR. This approach allowed us to speak with a uniform set of stakeholders at one time and present complementary research (saving stakeholders travel time and costs and allowing us to show how two separate ongoing projects can work together to enhance research capacity at the reserve and knowledge of local ecology).

    This meeting was attended by <u>stakeholders</u>:
    William C. Buie, Mississippi Department of Health
    Derek Whitney, Mississippi Department of Health
    Thomas Strange, GB NERR (GIS specialist)
    Kate Rose, NOAA National Data Development Center
    Jay McIlwain, Mississippi Division of Marine Resources
    Bradley Randall, Mississippi Division of Marine Resources
    Jeff Boyczuk, Chevron (industry located within the watershed)
    Tim Cook, City of Pascagoula
    David Groves, City of Pascagoula
    Coen Perrotti, Mississippi Department of Environmental Quality
    Scott Hereford, U.S. Fish and Wildlife – Mississippi
    Mark Woodrey, GB NERR (Biologist)

7. Integration Lead Walton and PI Carmichael initiated conversations with EPA and NERR personnel to clearly address potential overlap among projects at the NERR and define ways to complement our efforts while avoiding duplication.

- Has interaction with intended users brought about any changes to your methods for integration of intended users, the intended users involved, or your project objectives?
- How do you anticipate working with intended users in the next six months?

Stakeholders expressed interest in landscape changes (changes in river size and conversion from natural to urbanized landscapes) as part of modeling efforts, links between microbial inputs and wildlife vs. human waste vs. fish processing, and links between microbial counts and beach/swimming closures or fishing area closures. Most of these ideas are already part of our project, hence, we readily found common ground.

Project additions and benefits from the meeting:

a. All expressed interest in the project and desire to attend future meetings,
b. Several stakeholders directed us to existing (online) resources to aid land use map construction and groundtruthing (specifically the DEQ website and MS watershed characterization and ranking tool in which some data have already been analyzed and may be viewed or downloaded),
c. The suggestion was made to reach out to MS rotary clubs and similar groups for future outreach and presentations,
d. Bradley Randall of MS DMR also suggested we visit Belle Fountain Beach to search for Mercenaria (we could contact him for use of his oyster dredge) to aid our shellfish aging and comparison to ancient midden shells, and
e. David Groves of Pascagoula suggested reaching out to ecotour groups; we told him about our existing relationship with Capt. Katy (core stakeholder participant). He expressed interest in helping to develop this idea for the City of Pascagoula.

In the next 6 months, we plan to organize and hold a second stakeholder participant meeting at which stakeholders form both initial meetings can meet, and we can provide an update of progress to-date with informal discussion afterward.

C. Progress on project objectives for this reporting period:

- Describe progress on tasks related to project objectives for this reporting period.
- What data did you collect?
- Has your progress in this period brought about any changes to your methods, the integration of intended users, the intended users involved or the project objectives?
- Have there been any unanticipated challenges, opportunities, or lessons learned?

Field sampling

Sediment cores—In June 2011, graduate student Condon coordinated researchers from DISL, FDA, and the University of West Florida to collect 1 m long sediment cores from five subestuaries of the Grand Bay NERR, and a nearby wastewater outfall in Bayou La Batre, Alabama (*method change and integration of information from stakeholders: EPA and ALDMR**). Cores were sectioned and analyzed for *Clostridium perfringens*, stable isotopes, C and N, and will be radio-dated to determine historical influences of wastewater in this area.

*The collaboration with the University of West Florida to collect the cores was suggested after meetings by Carmichael and Condon with Just Cebrian (DISL) and John Lehter (EPA). As a result, the cores retrieved were much longer than hand-coring would have allowed, samples are much higher quality, and will be directly comparable with cores taken by the EPA project in February.

Oyster transplants—In early June, Condon transplanted oysters in up- and downstream locations in four subestuaries of the Grand Bay NERR and Bayou Chicot (city of Pascagoula).

- Oysters are sampled for growth, survival, stable isotopes and microbial analyses.

Environment data—Condon and technicians from the Carmichael Lab have sampled at transplant sites biweekly since the first week of June, collecting water, sediment, and oyster samples.

- Water samples are analyzed for chlorophyll, stable isotopes in suspended particulate matter (SPM), dissolved nutrients (N and P), and microbial content.
- Sediments are analyzed for particulate C and N, stable isotopes and microbial content.

YSI data were collected from four locations to complement NERR SWMP YSI data. Condon has liaised with Kim Cressman (GBNERR's SWMP coordinator) to collate YSI data from all nine locations (*Integration of information and participation by NERR staff enduser)*.

Condon contacted managers of the Jackson County and Bayou La Batre Utility Authorities to gain information on wastewater treatment plants to the west and east of the study area to facilitate and guide future source sampling efforts.

Midden shells—Condon continues to analyze ancient oyster shells from native American shell middens and shells found in historical sediment cores for changes in isotopic ratios indicating shifts in N sources and/or pollution.

Lab work and data analyses

Midden shells—Condon, Carmichael, and REU intern Amanda Jones, continued processing "practice" shell samples provided by Jackson (USM) from midden sites (Jun-Aug 2011).

Microbial data—Condon and Co-I Calci measured fecal coliforms, *E. coli*, *C. perfringens*, and male-specific coliphage (MSC) in water, sediments, and oysters at nine sites, for two time-points during the transplant period. The second sampling was completed in the second week of August, with the final (third) sampling planned at the end of September.

Results—Sediment Cores

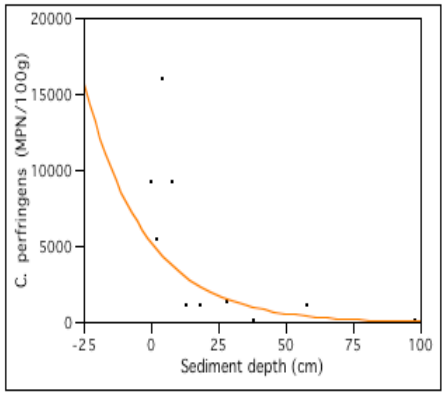
C. perfringens concentrations declined logarithmically with depth at all sites (Table 1). Logarithmic regressions were significant ($p < 0.05$) at all sites (Fig. 1). Cores taken near the Bayou La Batre wastewater treatment and fish processing plant outfalls had the highest level of *C. perfringens* in surface sediments, and the deepest penetration of viable *C. perfringens* spores. A few of the sites had higher *C. perfringens* concentrations in layers lower than the surface, which could be due to lower surface sediment bulk densities.

Table 1. *Clostridium perfringens* counts in surface and bottom sediments (58 cm) and regression statistics relating *C. perfringens* counts to core depth for six cores collected at sites in Grand Bay, Mississippi (shown in Fig. 1).

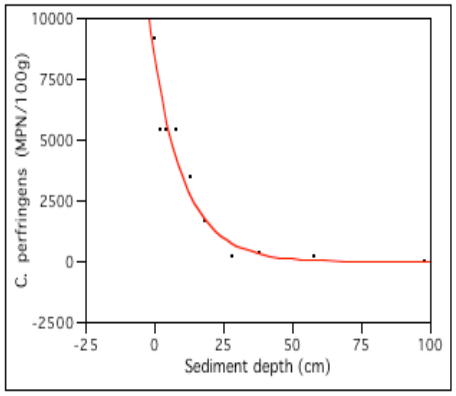
	C. perfringens (MPN/100g)		Regression Statistics		
Site	Surface	58 cm	Slope	r^2	p
Bayou la Batre 1	9200	1100	0.0438	0.575	0.011
Bayou la Batre 2	9200	220	0.0864	0.943	<0.0001
Bayou Heron	2400	<18	0.0927	0.875	<0.0001
Bayou Cumbest	640	<18	0.0855	0.566	0.0121
Bangs Lake	2400	<18	0.113	0.714	0.0021
Point aux Chenes	490	<18	0.08	0.492	0.0239

Fig. 1. Changes in *C. perfringens* counts with depth in the sediment for cores collected at six sites in Grand Bay, Mississippi. Regression statistics are given in Table 1.

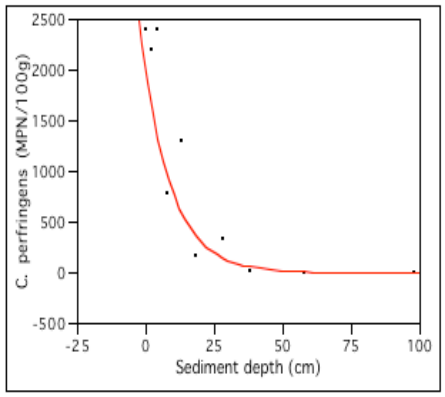
Bayou la Batre Core 1



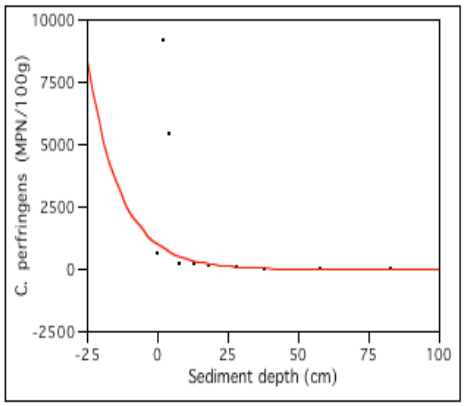
Bayou la Batre Core 2



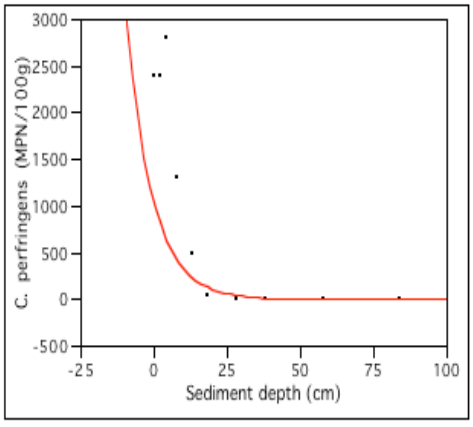
Bayou Heron



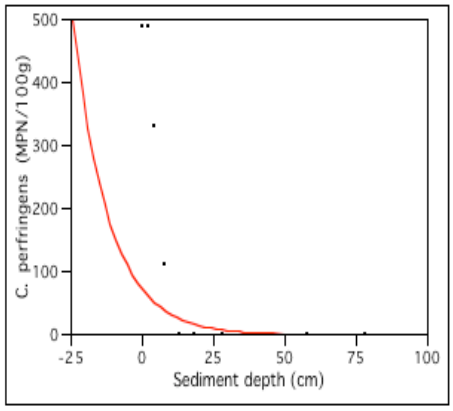
Bayou Cumbest



Bangs Lake



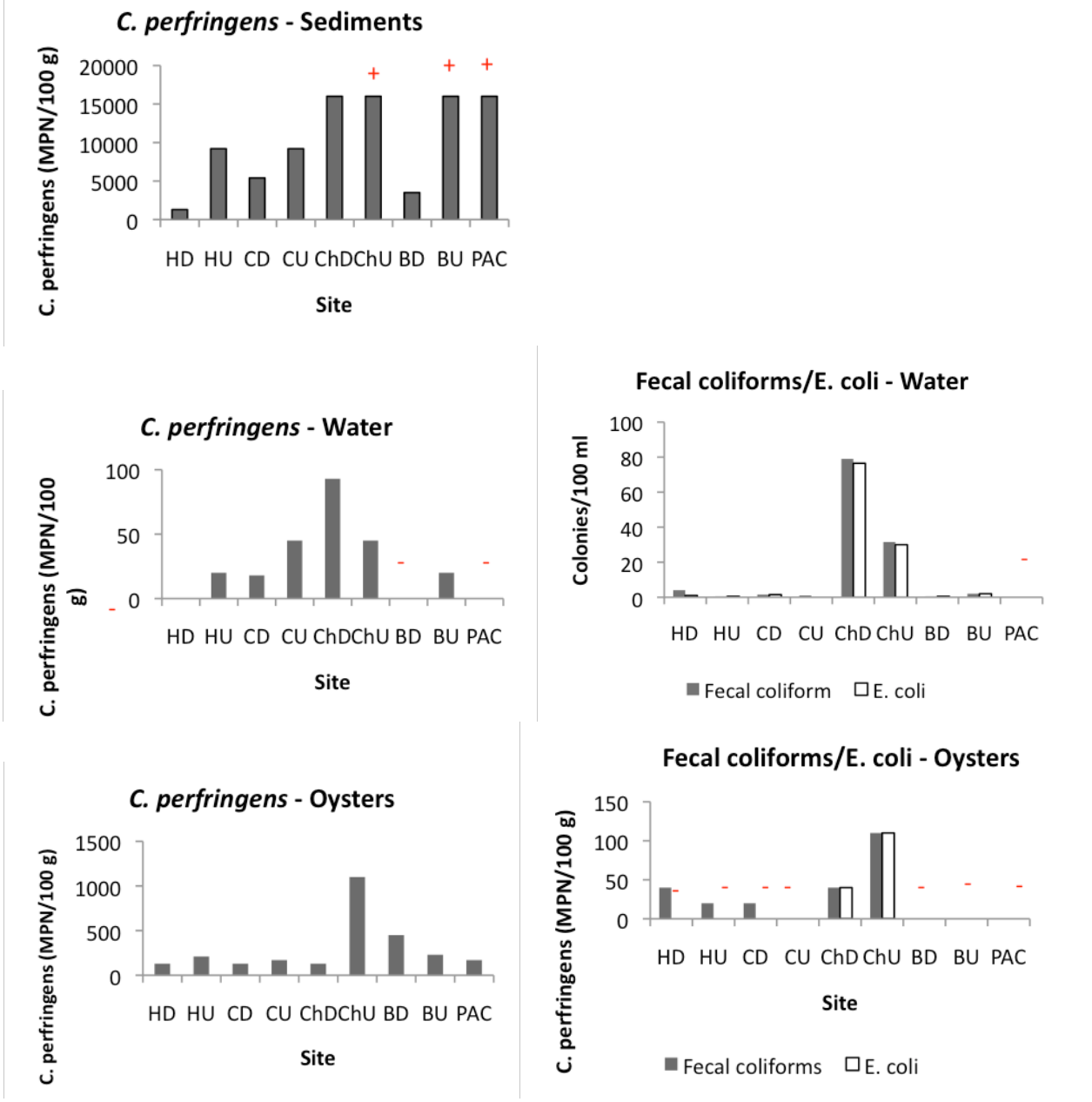
Point aux Chenes Bay



Results—Surface sediment, water, oysters

High loads of *C. perfringens* were found at Bayou Chicot upstream and downstream and at Point aux Chenes Bay (Fig. 2). For all bayous, downstream sites had lower levels of *C. perfringens* than upstream sites within a bayou. High *C. perfringens* were found in oysters from Bayou Chicot upstream and in water at Bayou Chicot downstream. The Bayou Chicot up- and downstream sites also had water and oysters that were positive for fecal coliforms and *E. coli*, while other sites were negative for *E. coli* in oysters and below detection limits for *E. coli* in water.

Fig. 2. *C. perfringens* in surface sediments and *C. perfringens* and fecal coliforms/E. *coli* in water and oysters from up (U) and downstream (D) sites in Grand Bay, MS. Bayou Heron (H), Bayou Cumbest (C), Bayou Chicot (Ch), Bangs Lake (B), and Point aux Chenes (PAC).



Technical training

Co-I Calci (FDA) and student Condon continued training and developing laboratory techniques for the microbiology component of the project. At the FDA Gulf Coast Research Lab on Dauphin Island, they worked on techniques for enumerating *E. coli*, fecal coliform, and male-specific coliphage (MSC) in water, surface sediments, and oysters, and the iron-milk method for *Clostridium perfringens* detection in sediment cores and surface sediments.

FDA provided logistical support in fabricating an effective core catcher for the sediment corer.

PI Carmichael and technician trained Condon and REU intern, Amanda Jones, on shell grinding and acidification techniques in the Carmichael lab for application to midden shells provided by Jackson (USM) (Feb 2011).

FDA and PI Carmichael reviewed and provided comment on preliminary microbial data collected by Condon.

Land-use modeling

We applied medium-resolution Landsat-5 TM satellite imagery (30 m, except for thermal infrared band which is 120 m) obtained on 29 February 2008 to classify urban area in the Grand Bay NEER and its surrounding areas within 10 km to the Grand Bay NERR boundary (Fig 3). We utilized unsupervised classification based on ISODATA algorithm, aided by the high resolution imagery QuickBird obtained on 28 February 2008 as a visual check.

Due to the Landsat-5 TM imagery's medium spatial resolution and the nature of high heterogeneity in urban area, we could not use the classification as the final product. However, the medium-resolution urban area facilitates land classification using high-resolution imagery since it can increase the high-resolution classification accuracy and speed when it is used as an urban mask. The mask is reliable since Landsat-5 TM imagery has 7 spectral bands (blue, green, red, near-infrared, middle-IR, thermal IR, shortwave IR).

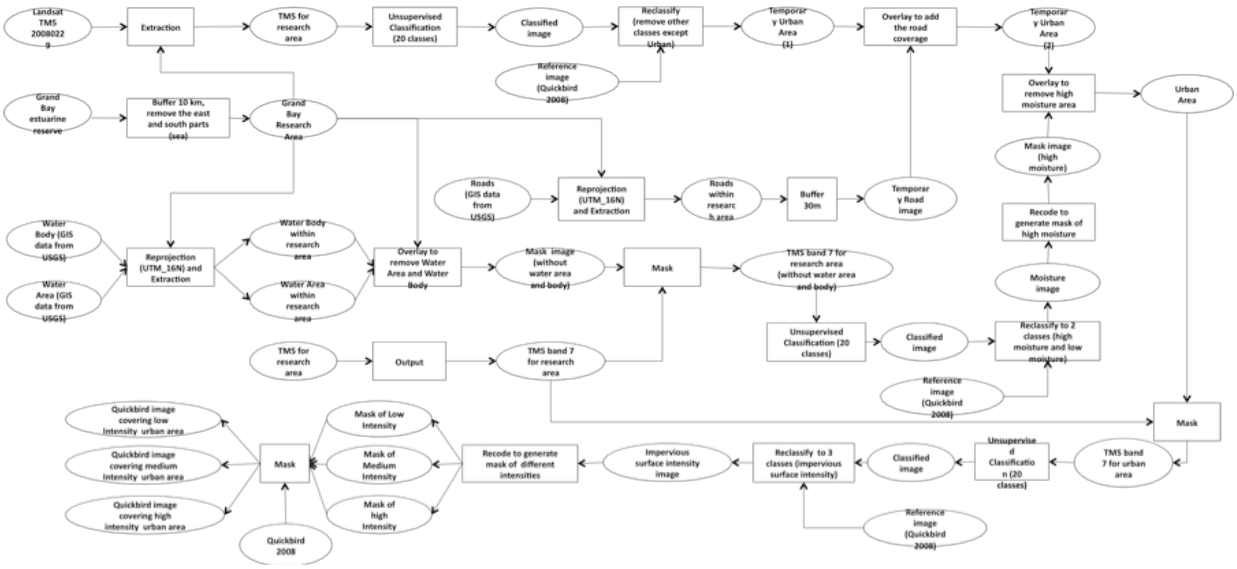


Fig. 3. Model flow diagram to classify urban areas using medium-resolution Landsat-5 TM satellite imagery aided by high-resolution Quickbird satellite image.

High resolution Quickbird imagery has four spectral bands: blue, green, red and IR (useful for vegetation detection). With the auxiliary information from Landsat-5 TM imagery, we can make good use of Quickbird's high spatial resolution (3 m) to capture the heterogeneity in urban area. We are in the process of applying the high-resolution QuickBird satellite imagery to classify impervious area using the urban area classified from the Landsat-5 TM imagery as a mask.

The area classified as urban using Landsat-5 TM imagery was 71.75 km^2, 24% of the entire study area (Fig. 4A). This area was comprised of 18.57 km^2 of high-intensity urban area (defined as a mixture of impervious area, low grass and barren), 19.62 km^2 of medium-intensity urban area (defined as a mixture of less impervious area, high grass, and trees), and the 33.56 km^2 of low-intensity urban area (defined as shallow water and pools) (Fig. 4B).

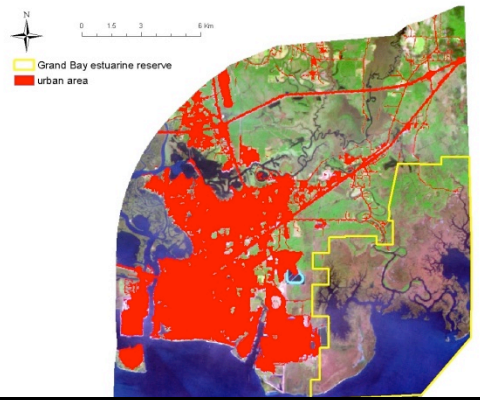


Figure 4A Urban coverage for Bayou Chicot (71.75 km^2) classified in red using Landsat-5 TM imagery (background: Landsat imagery).

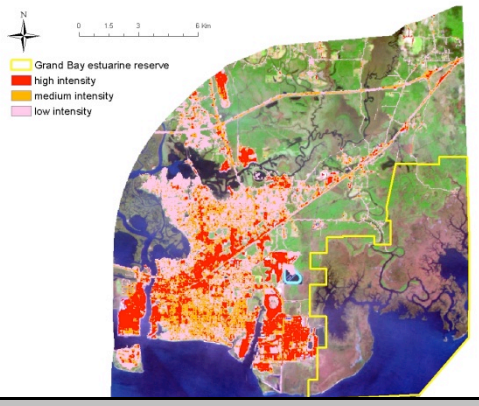


Figure 4B The urban coverage with three different intensities (18.57, 19.62, and 33.565 km^2 from high to low intensities) (background: Landsat imagery).

In all, land-use data compiled includes:

- Habitat map of Grand Bay NEER in 2004 from GIS specialist Tom Strange at the Grand Bay NERR.
- Quickbird imagery including Grand Bay NERR in 2008 from Dr. Greg Carter at the Gulf Coast Geospatial Center.
- We have spoken to Dr. Cebrian at DISL regarding the sub-basin boundary file and the possibility of sharing the data once the survey is done.
- GIS files of roads and hydrology from Mississippi Automated Resource Information Center.

There have been no changes in methods, except the potential for using the existing watershed boundaries delineated by Cebrian and EPA.

The greatest challenge in analyzing these data has been delineating the highly heterogeneous urban area, the water bodies (easy to mix with building shadows) nearby, and the barren land on wetland areas due to the complexity.

To resolve these issues and increase the classification accuracy, we applied masks based on the roads and water bodies, and the moisture index derived from Landsat images (Band 7). Band 4, useful for vegetation identification, is sometimes combined with Band 7 for the moisture index,

but it did not work here since most urban areas have very good vegetation coverage, so we only applied Band 7.

- What are your plans for meeting project objectives for the next six months?

  We will continue collecting and processing data from transplanted oysters, shell middens, and sampling native bivalves, and we continue regular measurements of estuarine attributes at each site. We will continue stable isotope and microbial analyses on sediment, water, and oyster samples, begin measuring bivalve growth and survival on transplanted oysters and based on native collections and core captures. We will also be extracting organic material from ancient bivalve shells (1000-2000 years old) and analyzing stable isotopes of this material.

  We plan to continue land-use research and modeling efforts as described, including improvement of the urban classification by exploring the integration of texture information. We will develop the sub-pixel algorithm using a regression tree based on high resolution Quickbird and medium-resolution Landsat imagery to derive the percentage of impervious areas for each Landsat pixel (30 m) in the study region and apply the algorithm to the current Landsat imagery, which we will validate by site visits and ground-truthing. Once the algorithm has been validated, we will apply it to Landsat imagery in 1970s, 1980s, 1990s, and 2000s. We will predict the land use pattern in 2020 based on the changing rates and patterns derived from the time-series land cover maps, and other environmental and social variables.

  We will continue operation and maintenance of the Facebook page and Google discussion board, make at least one public or scientific presentation to share data from the project, as well as continue data analyses and student and technician training.

D. Benefit to NERRS and NOAA: List any project-related products, accomplishments, or discoveries that may be of interest to scientists or managers working on similar issues, your peers in the NERRS, or to NOAA. These may include, but are not limited to, workshops, trainings, or webinars; expert speakers; new publications; and new partnerships or key findings related to collaboration or applied science.

See results above from microbial and land-use modeling efforts.

PI Carmichael and Co-I Ruple attended a project update and stakeholder meeting for an ongoing EPA based project led by Dr. J. Cebrian (DISL) to share information about our complementary projects. Data from this other project will inform modeling efforts by Co-I Wu for this project, leverage data collection and enhance outputs from both projects (already evidenced by our vibracoring efforts), and seed future collaborations at the Grand Bay NERR (Aug 2011). *At this meeting PIs Carmichael and Cebrian discussed with EPA an idea to bring the outputs of both projects together to develop a management tool for endusers to assess land-use and urban development projects in context of estimated nitrogen concentration and source changes to watersheds and receiving estuaries. A proposal is in discussion for the future.*

Condon submitted an abstract for this work to be presented at the Coastal and Estuarine Research Federation (CERF) conference in Daytona Beach, Florida in November 2011. Very few studies have examined *C. perfringens* spores in historical sediment cores, and this is likely the first study to pair analysis of *C. perfringens* concentrations with shifts in stable isotopes (organic N and C sources) on a decadal scale. The microbiological work on these sediment samples is finished; stored sediment samples are currently being processed for stable and radio-isotopes.

Thanks to a fellowship program between DISL and the FDA, leveraging funds from the NSC project, Condon was able to conduct the more expensive but also more thorough vibracoring method for sediment coring, which was not included in the original proposal. As a result, the cores retrieved were longer and samples are higher quality than possible with hand-coring, and data will be directly comparable to data from cores taken by the EPA project in February.

Based on the success of this collaborative research project, in July, FDA administrators used Condon's progress as an example of positive outputs from and an impetus to continue funding for the DISL/FDA joint fellowship program.

E. Describe any activities, products, accomplishments, or obstacles not addressed in other sections of this report that you feel are important for the Science Collaborative to know.

Co-I Wu hired a post-doc position, which will be supported by this project and a related project. Dr. Chongfeng Guo has taken the lead on the land-use change analysis. Dr. Guo prepared the figures included for the land-use modeling results to-date.